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Animal Study on Early Tooth Movement after Ramus Osteotomy

하악지 골절단술 이후의 초기 치아이동에 관한 동물 연구

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서울대학교 대학원

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-ABSTRACT-

Animal Study on Early Tooth Movement after Ramus Osteotomy

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*(Directed by Professor **Tae-Woo Kim**, DDS, MSD, PhD)*

Introduction: The purpose of the present study was to observe whether mandibular ramus osteotomy, remote from dental apparatus, has any cellular or molecular influences on tooth movement at an early stage in a rabbit model.

Methods: Eighteen male New Zealand white rabbits (4.0 kg body weight) were allocated into 3 groups: Group 1 – tooth movement without operation (n = 6), Group 2 – tooth movement with sham operation (n = 6) and Group 3 – tooth movement with ramus osteotomy (n = 6). In all of the three groups, only the right teeth were moved with NiTi (Nickel Titanium) closed coil spring. The amount of tooth movement was measured by the distance only between the right incisor and the right first molar. At 5 (T1), 7 (T2), and 14 days (T3) two rabbits were sacrificed from each group. The numbers of osteoclasts were counted on vertical sections of alveolar bone in four areas including the compression sides of the right loaded teeth and the

left unloaded teeth. Relative quantitation of mRNAs by real time reverse transcription-polymerase chain reaction (PCR) was performed for receptor activator of nuclear factor κB ligand (RANKL), osteoprotegerin (OPG), interlukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)-α at T2 and for RANKL and OPG at T3 in four areas of alveolar bone as for osteoclast counting.

Results: Although Group 3 manifested the highest distance of tooth movement (total 5.05 mm), there was no significant difference among groups. There was no significant difference in the number of osteoclasts for the right incisor and the right first molar in all three groups at T1, T2, and T3. However at T1, there was a marginally significant difference only for the right incisor in all groups ($p = 0.052$). On the left side there was no significant difference among groups for the incisor and first molar, respectively. At T3, there was a significant difference in RANKL for the right first molar (Group 3 > 1 > 2, $p < 0.05$). At T2 and T3, there was no consistent results in level of OPG in all groups. At T2, there was no significant difference in IL-1α, IL-1β, IL-6, TNF-α in all four sites.

Conclusions: The present study suggests that the orthognathic surgical regimen (mandibular ramus osteotomy) has some influences on molecular change in alveolar bone adjacent to the osteotomy site at an early stage of tooth movement in a rabbit model.

Key Words : Ramus osteotomy, tooth movement, osteoclast

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I. Introduction

Harold Frost recognized that surgical wounding of osseous hard tissues results in striking reorganizing activity of cells and tissues adjacent to the site of injury. He collectively termed this cascade of physiologic healing events as a regional acceleratory phenomenon (RAP)[1,2].

In rats, evidence of RAP was first observed after 10 days of healing, and there was almost complete recovery after 120 days; it was suggested that RAP in humans began within a few days of surgery, typically peaks in the first and second month, and might take from 6 months to more than 24 months to subside[3]. The phenomenon is a complex physiologic process with dominating features involving accelerated bone turn-over and decreases in regional bone density. They characterized the initial phase of RAP as an increase in cortical bone porosity because of increased osteoclastic activity, and they surmised that RAP might be a contributing factor to increased mobility of the teeth and to accelerated tooth movement.

Several clinical studies showed that the tooth movement was accelerated in conjugation with corticotomy of human alveolar bone[4,5,6,7]. They recommended completing the major tooth movements in 3 to 4 months and finishing of most tooth movement in less than 12 months.

Other clinical study reported that after orthognathic surgery in human, RAP activated in fracture healing accelerated the tooth movement[8]. This clinical study showed that the tooth movement was accelerated and the serum alkaline phosphatase and C-terminal telopeptide of type I collagen (ICTP) level, which is a bone resorption metabolite of type I collagen in bone, significantly increased in the first week to the third month postoperatively in twenty two adults. The authors suggested that the phenomenon of postoperatively accelerated orthodontic tooth movement was because of the increase in osteoclastic activities and metabolic changes in the dentoalveolus caused by orthognathic surgery.

The author observe accelerated tooth movement after orthognathic surgery in a clinical situation. However, there have been few studies of tooth movement after orthognathic surgery at the cellular and tissue levels.

Therefore, the purpose of this study was to observe whether mandibular ramus osteotomy has any influence on tooth movement at an early stage for two weeks, which was measured in distance and also observed at the cellular and tissue levels.

II. Review of literature

Frature healing and regional acceleratory phenomenon

Fracture healing is a multistage repair process that involves complex yet well-orchestrated steps that are initiated in response to injury, resulting eventually in the repair and restoration of function[9]. The process normally proceeds in successive stages named the fracture, granulation(cartilage), and modeling / remodeling stages.

Each process requires far more than just osteolasts[10~19].

During the healing process, the pathway of normal embryonic development is recapitulated with the coordinated participation of several cell types[20]. All four components involved in the injury site, including the cortex, the periosteum, the bone marrow, and the external soft tissues, contribute in the healing process at different extent, depending on multiple parameters present at the injured tissue such as growth factors, hormones and nutrients, pH, oxygen tension, the electrical environment and the mechanical stability that has been obtained[21,22].

The processes are initiated by an immediate inflammatory response, which leads to the recruitment of mesenchymal stem cells and the subsequent differentiation into chondrocytes that produce cartilage and osteoblasts,

which form bone. After cartilage matrix is produced, it is transformed from mineralized cartilage to bone. This primary bone formation is followed by remodeling, in which the initial bony callus is reshaped by resorption and then by secondary bone formation to restore the functional load-bearing anatomical structure[17]. The biological processes driving these stages are regulated by cell signaling molecules that can be categorized into three groups: (1) pro-inflammatory cytokines, (2) transforming growth factor-beta superfamily (TGF-β) members, and (3) angiogenic factors. The biological activities of these molecules trigger overlapping biological processes and coordinate interactions between differing cell populations[9,16,17].

At final stage complete replacement of the callus, which is mineralized granulation (cartilage) tissue, with functionally competent lamellar bone by remodeling multicellular unit consumes one to four years. But it proceeds quickly during the first one-third of the replacement and slows progressively in the last two-thirds. It is known as regional acceleratory phenomenon (RAP)[1,2]. The RAP does not seem to provide new processes, but by increasing the rapidity of the other healing stages, it makes healing occur two to ten times more quickly than otherwise. Since a RAP normally occurs after a fracture, arthrodesis, osteotomy, or bone grafting operation[23,24], one appreciates its existence in nearly all cases (more than 97% of bone injuries). RAP occurring in jaw bone could be induced by flap surgery[3], corticotomy[7,25,26], and even by a non-surgical procedure such as orthodontic tooth movement[25,27]. It begins within a few days of the fracture, typically peaks at one to two months, and may take six to more than 24 months to subside[1,3].

Cellular and molecular process in fracture healing

After bone fracture an immediate inflammatory response begins, which leads to the recruitment of mesenchymal stem cells and the subsequent

differentiation into chondrocytes that produce cartilage and osteoblasts, which form bone. The inflammatory phase is associated with the formation of a hematoma, recruitment of mesenchymal stem cells, cell proliferation, and the initiation of chondrogenesis, followed by vascular ingrowth and neo-angiogenesis. Interleukins-1 and 6 (IL-1 and IL-6), and tumor necrosis factor (TNF-α) play a role in initiating this repair cascade. Tumor-derived growth factor (TGF-β), platelet-derived growth factor, and bone morphogenic protein (BMP-2) expression increase to initiate callus formation[9,16,17,18]. BMP-5 and BMP-6 and other members of the TGF-β superfamily are constitutively expressed suggesting regulatory effects on both intramembranous and endochondral ossification[28]. More recently it has been suggested that growth differentiation factor (GDF-8) has a role in controlling cellular proliferation[28]. Angiopoietins and vascular endothelial growth factors (VEGFs) are induced to stimulate vascular ingrowth from vessels in the periosteum essential for the fracture repair process to progress.

The primary bone formation phase displays the most rapid osteogenesis, involving (1) bone cell recruitment and woven bone formation; (2) chondrocyte apoptosis, osteoclast recruitment, and mineralized cartilage resorption; and (3) continued neo-angiogenesis. A rise in TNF-α, receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (MCSF), is associated with mineralized cartilage resorption, the recruitment of mesenchymal stem cells and induces apoptosis of hypertrophic chondrocytes[9]. BMP-3, BMP-4, BMP-7, and BMP-8 rise in association with the resorption of calcified cartilage and promote recruitment of cells in the osteoblastic lineage. VEGFs are upregulated to stimulate neoangiogenesis[9,16,17,18]. Secondary bone formation and bone remodeling are associated with the reconstitution of normal marrow. IL-1 and IL-6 again rise in association with bone remodeling, whereas RANKL, MCSF, and TGF-β display diminished

levels[9,16,17,18].

Among those signaling molecules we focus on the cytokines (TNF-α, IL-1, IL-6, RANKL, OPG) and transmembrane protein (RANK) associated with the action of osteoclast directly or indirectly.

IL-1, IL-6 and TNF-α have been shown to play a role in initiating the repair cascade. They induce a downstream response to injury by recruiting other inflammatory cells, enhancing extracellular matrix synthesis, and stimulating angiogenesis[29].

TNF-α, a multifunctional cytokine mainly produced by activated macrophages, has numerous functions. The soluble TNF-α (mature protein) is released, under physiological conditions, by a proteolytical cleavage of the precursor pro TNF-α, a type II transmembrane protein. TNF-α is one of the most potent osteoclastogenic cytokines produced in inflammation[30,31,32].

IL-1, which is a pleiotropic cytokine produced by activated macrophages and endothelial cell, makes osteoclasts induced by TNF-α to have the capacity to form resorption pits on bone[33].

The main sources of IL-6 in bone are osteoblastic cells, stromal cells and not osteoclastic cells. However, the main activity of IL-6 on bone is its effect on osteoclastogenesis and bone resorption. IL-6, like others resorptive agents, stimulates osteoclast activity and bone resorption by an indirect mechanism, increasing interactions between osteoblasts and osteoclasts. IL-6 stimulates osteoclast-like formation in long-term human marrow cultures by inducing IL-1 release, and mediates the stimulatory effects of TNF. And it enhances bone resorption by increasing the pool of osteoclastic progenitors and their differentiation into mature osteoclasts[34,35].

TNF-α, IL-1 and IL-6 stimulate osteoclast differentiation in a synergistic fashion. In addition, IL-1, TNF-α, and IL-6 are known to control their own expression. These cytokines may not only regulate osteoclastogenesis by stromal cells, but also act directly on osteoclasts and their precursors. In vivo, the rate of osteoclastogenesis responds to the sum of all activating and

inhibitory signals, and the entire process is subjected to rapid modulation[36].

Osteoprotegerin (OPG) and RANKL, synthesized by stromal cells / osteoblasts, have been identified as the two principal cytokines of the osteoclastic differentiation and activation[37,38,39].

RANKL is a protein of 317 amino-acids which belongs to the TNF super-family and whose mRNA is largely expressed in bone, bone marrow and lymphoid tissues. The predominant role of this cytokine in bone physiology is the stimulation of osteoclastic differentiation/activation and the inhibition of osteoclast apoptosis[39].

RANKL in association with macrophage-colony stimulating factor (M-CSF) is necessary and sufficient for the complete differentiation of osteoclastic precursors into mature osteoclasts.

RANK, localized at the cell surface of mature osteoclasts and osteoclastic precursors, is a transmembrane protein of 616 amino-acids which belongs to the TNF receptor superfamily, the third protagonist and the natural receptor of RANKL[31,39].

Binding of RANKL to RANK stimulates (1)differentiation of osteoclastic precursors into mature osteoclasts, and (2)activation of mature osteoclasts. The RANK activation by RANKL is followed by its interaction with various intracellular components of the signaling pathway.

The dominant outcome of TNF-α, IL-1 and IL-6 is a net increase in RANKL activity[30,40]. TNF-α, IL-1 and IL-6 can act independently or synergistically with RANKL, and they also stimulate RANKL production by osteoblastic cells and thus bone resorption.

OPG, which is synthesized as a protein of 401 amino-acids, acts as a decoy receptor for RANKL and down-regulates the RANKL signaling through RANK. It represents an antagonist endogenous receptor that neutralizes the biological effects of all forms of RANKL and thus acts as an inhibitor of bone resorption. The biological effects of OPG on bone cells include inhibition of terminal stages of osteoclast differentiation, suppression of mature

osteoclast activation, and induction of apoptosis[30,41].

Finally, bone remodeling appears to be mainly controlled by the balance of RANK / RANKL / OPG triad.

Cellular and molecular process in tooth movement

Orthodontic forces induce an aseptic inflammatory response. During early stages of tooth movement, there is an increase in vascular permeability and cellular infiltration of leukocytes[42]. Cells of the osteoblast lineage play a pivotal role in bone remodeling, a process that involves interactions between osteoblasts and osteoclasts, systemic hormones, cytokines, and growth factors. Bone resorption is crucial to orthodontic treatment, by removing alveolar bone from the path of the moving dental root. In this cell-mediated process, the appearance of osteoclasts is considered to be the requisite first step[42]. However, it is unclear whether these cells arise from activation of mature osteoclasts already in the PDL or from the proliferation of stem cells in remote or local hemopoietic tissues[43]. According to the widely accepted hypothesis by Mundy and Roodman[44], osteoclasts are derived from stem cells in hemopoietic organs, and granulocyte-macrophage colony-forming units are the earliest identifiable precursors of osteoclasts. The proposed pathway can be outlined as follows: granulocyte — macrophage colony-forming units — promonocyte — early preosteoclast — late preosteoclast — osteoclast.

Several lines of evidence exist for the fact that the immediate precursors of osteoclasts—the late preosteoclasts—are present in the PDL and are activated or transformed to mature osteoclasts after orthodontic mechanotherapy. Roberts and Ferguson[45] found that osteoclast numbers per unit bone surface area show a peak level about 50 hours after orthodontic force application. Additionally, new osteoclasts reach the PDL from hemopoietic organs via the blood circulation, and from alveolar bone marrow cavities, during the orthodontic treatment period, which can last 2 to 3 years.

The bone-resorption cascade involves a series of steps directed toward removing both the mineral and the organic constituents of bone matrix by osteoclasts[46].

Osteoclast is activated by local and systemic factors, and production of hydrogen ions (that dissolve the mineral) and proteolytic enzymes (that degrade the organic matrix) in the hemivacuole (localized environment) under the ruffled border of the cell[47]. In addition to this classic concept, recent findings have proposed a new concept, in which osteoblasts can activate osteoclasts through cell-to-cell contacts[48]. The osteoclasts thus activated produce hydrogen ions and proteolytic enzymes in the ruffled border of the cell. It has been proposed that these hydrogen ions are generated in the cell by the enzyme carbonic anhydrase, present in the cytoplasm close to the ruffled border[49]. Osteoclasts ultimately undergo apoptosis, characterized by nuclear and cytoplasmic condensation, and fragmentation of nuclear DNA into nucleosomal sized units[50].

Once the osteoclasts finish their work of bone removal, there is a "reversal phase," when mononuclear cells, which might be of macrophage lineage, are seen on the bone surface[42]. The end of bone resorption and the start of bone formation occurs through a coupling mechanism, which ensures that an equivalent amount of bone is laid down after the previous resorption phase. Whether activation of osteoblasts begins simultaneously with osteoclast recruitment or at a later stage during lacunar development is still controversial.

Cytokines are extracellular signaling proteins that act on nearby target cells in low concentrations in an autocrine or paracrine fashion in cell-to-cell communications.

During early stages of tooth movement, migrated immune cells along with native cells such as fibroblasts and osteoblasts produce inflammatory cytokines that include lymphocyte- and monocyte-derived factors, colony-stimulating factors, growth factors, and chemotactic factors[51,52]. High

concentrations of inflammatory cytokines such as IL-1, IL-2, IL-3, IL-6, IL-8, TNFα, interferon-γ (IFNγ), and osteoclast differentiation factor have been found in the gingival crevicular fluid surrounding moving teeth[53,54,55].

The role of cytokines during tooth movement is not clear. It has been suggested that cytokines and other inflammatory markers, such as prostaglandin E2[56], may activate bone remodeling characterized by bone resorption in the compression region and bone deposition in the tension region of the periodontal ligament (PDL)[54]. One possible mechanism through which inflammatory cytokines may affect bone remodeling is through recruitment of osteoclast precursors from the circulation, their maturation and activation. the rate of tooth movement correlates with the efficiency of bone remodeling.

The effect of cytokine expression on bone remodeling is important in the alveolar process. Studies of knockout mice deficient for TNFα receptors showed a slower rate of tooth movement in response to orthodontic forces[57].

As mentioned above, cytokines that were found to affect bone metabolism, and thereby orthodontic tooth movement, IL-1, IL-2, IL-3, IL-6, IL-8, TNFα, IFN-γ and osteoclast differentiation factor (ODF). The most potent among these is IL-1, which directly stimulates osteoclast function through IL-1 type 1 receptor, expressed by osteoclasts[42].

Since bone remodeling occurs at discrete sites throughout the skeleton, osteoblast-derived cytokines are ideally placed to regulate or modify the action of other cell types in bone[58]. The first cytokine shown to play a role in bone turnover was IL-1[59,60]. Not long afterwards, the TNFs were shown to stimulate bone resorption and inhibit bone formation in vitro[61], and subsequent studies have implicated numerous cytokines including IL-6.

Meikle et al.[62] proposed that cytokines such as IL-1 produced locally by mechanically activated cells were responsible for mediating both the resorptive and formative phases of connective tissue remodeling. The

immunolocalization of IL-1 β in the periodontal tissues of cat canine teeth following the application of a tipping force provided the first experimental evidence in support of this hypothesis[63]. Clinical studies have since shown that IL-1 β , TNF- α , IL-6, and epidermal growth factor are all elevated in gingival crevicular fluid collected from patients during the early phases of orthodontic tooth movement[64,65,66]. These findings have since been confirmed by numerous other investigations.

After many attempts to purify, characterize, and sequence an osteoclast-activating factor using biochemical methods, a cell-surface protein that was found to be identical to receptor activator of nuclear factor κ B ligand (RANKL) was simultaneously discovered by two research teams[38,67] using molecular techniques. RANKL and its receptor RANK expressed on osteoclasts and their precursor cells turned out to be the molecular determinants of osteoclast formation and function. Cell - cell signalling by RANKL is essential for the induction of osteoclast differentiation. PTH and other systemic hormones as well as cytokines, such as IL-1, TNF-α, and IL-6, stimulate bone resorption by their ability to upregulate RANKL expression by osteoblasts / stromal cells. Another cytokine osteoprotegerin (OPG)[37] also produced by osteoblasts/stromal cells acts as an inhibitor of osteoclast function by competing with RANKL for the membrane receptor RANK. Evidence is emerging which suggests that RANKL and OPG produced by PDL fibroblasts and osteoblasts play important roles in regulating connective tissue turnover and bone resorption during orthodontic tooth movement.

Kanzaki et al.[68] reported recently that OPG gene transfer to periodontal tissues inhibited RANKL-mediated osteoclastogenesis and inhibited experimental tooth movement in rats. The number of reports cited above makes it clear that bone remodeling, particularly bone resorption, is regulated by cytokines released in response to the orthodontic force.

Systemic acceleratory phenomenon

Teixeira et al.[69] reported that stimulating the expression of inflammatory cytokines, through small perforations of cortical bone far away from teeth, increases the rate of bone remodeling and tooth movement in 48 rats. Micro-computed tomography, light and fluorescent microscopy, and immunohistochemistry demonstrated higher numbers of osteoclasts and bone remodeling activity in the flap and perforation group, accompanied by generalized osteoporosity and increased rate of tooth movement. They demonstrated that the increase in bone remodeling rate was not limited to the area of the loaded tooth, but extended to the tissues surrounding adjacent teeth, and the perforations do not need to be close to the tooth to be moved to accelerate the rate of movement.

The rate of remodeling in the region of a bone defect exceeds normal tissue activity. It was Frost who described this reaction as a regional acceleratory phenomenon (RAP)[1,2]. Those local healing process in the rat tibia with a bur hole defect resulted in a significant increase in mineralizing surface, mineral apposition rate, and bone formation rate in both femora and the fourth lumbar vertebra[70]. It was suggested that the regional enhancement of bone formation result in a systemic impact on bone metabolism, that is a systemic acceleratory phenomenon (SAP) accompanied the RAP and SAP affected only the cancellous, but not the cortical bone compartment. And they reported that SAP is associated closely with the occurrence of woven bone during the formation phase of the healing process, and that woven bone formation plays a pivotal role in the mediation of SAP.

III. Materials and methods

Experimental animals

Eighteen male New Zealand white rabbits (*Oryctolagus Cuniculus*), which had been bred for about 5 ~ 7 months to body weight of 4.0 kg in the uniform condition, were used. They were allocated into 3 groups: Group 1 – tooth movement without operation group (n = 6), Group 2 – tooth movement with sham operation (n = 6) and Group 3 – tooth movement with ramus osteotomy (n = 6).

This study was approved by the Institutional Animal Care and Use Committee and the number of approval is *SNU-120612-3*.

Measurement of tooth movement

For tooth movement, the mandibular right incisor and the first molar were connected with Nickel Titanium (NiTi) closed coil spring (509-22, Closed Coil Spring-Tension Spring®, Tomy Inc., Tokyo, Japan) in each subject, which was calibrated to traction force of 100 gm (**Figure 1**). We measured the distance between only the right incisor and first molar at the cement-enamel junction level with digital vernier calipers (Point Caliper®, Mitutoyo corp., Tokyo, Japan).

On the second day, sham operation only on the right side in Group 2 (n = 6) and vertical ramus osteotomy only on the right side in Group 3 (n = 6) were given, respectively. At 5 (T1), 7 (T2), and 14 days (T3) two rabbits in each group were sacrificed. We only measured the distance between the right incisor and first molar at T1, T2, and T3 (**Figure 2**).

Ramus osteotomy in Group 3

After mixing xylazine hydrochloride (Rompun®, Bayer Korea, Seoul, Korea) and tiletamine hydrochloride (Zoletil 50®, Virbac, Carros, France) in the

ratio of 2:3, intravenous injection into the marginal ear vein was gently done for general anesthesia.

The vertical incision line was about 30 mm long and located on only the right post-ramal area. After dissecting and opening the right ramus, the osteotomy was done with low-speed handpiece from post-condylar notch to the postero-inferior area of the mandibular body in parallel with the anterior border (**Figure 3**).

The separated bones were then fixed in the middle of the osteotomy line with a 2-hole mini-plate (16-ST-012, Leforte System Bone Plate®, Jeil Medical Corp., Seoul, Korea) and two mini-screws (16-MD-006, Leforte System Bone Screw®, Jeil Medical Corp., Seoul, Korea) (**Figure 3**). After layer suture, an antibiotic (Cefazolin®, Chong Kun Dang Phamaceutical Corp., Seoul, Korea) was intramuscularly injected.

Specimen preparation

At T1, T1 and T3, two subjects in each group were sacrificed with potassium chloride injection (Jeil Pharmaceutical Corp., Daegu, Korea) under general anesthesia. On the mesial side of the mandibular right and left first molars and distal side of the mandibular right and left incisors (total four areas), the bone with the part of the roots was cut out vertically. Each block bone was divided into half and allocated for microscope observation and real time polymerase chain reaction (PCR) respectively. At each day of sacrifice, total 48 specimens were made from six rabbits. 24 specimens were fixed for microscopic observation and 24 ones for real time PCR.

Microscopic observation and semi-quantitation of osteoclast

After block bones for microscopic observation were fixed in formalin, incubating solution was prepared as follows; freshly 0.2 ml 4% sodium nitrite solution was added to 38 ml of 0.1 M acetate buffer (pH 5.0),

followed by 2.0 ml of Naphtol AS-BI phosphoric acid solution (Sigma-Aldrich Corp., St. Louis, MO, USA). The pH of the final solution was 5.0. All tissue sections were incubated in an incubator at 37℃ for 3 days, when all multinucleated cells were stained by acid phosphatase substrate made from incubating solution. After incubation the slides were washed 3 times in distilled water, and not counterstained, air dried and covered with coverslips. Under 100 times magnificent power, three views of slide where the stained multinucleated cells could be seen the most were randomly chosen and all the stained multinucleated cells in touch with alveolar bone were counted. Each mean data was recorded and statistically analyzed (**Figure 4**). After a week, we repeated the same procedure.

Relative Quantitation of mRNAs by Real Time Reverse Transcription-PCR (Polymerase Chain Reaction)

We quantified mRNAs of RANKL, OPG, IL-1α, IL-1β, IL-6, and TNF-α obtained from the subjects at T2 (7^{th} day sacrifice). And mRNAs of RANKL and OPG obtained from the subjects at T3 (14^{th} day sacrifice) were quantified.

Total RNA was prepared by RNeasy mini kit® (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and cDNA was synthesized from 2 μg of total RNA by reverse transcriptase (Superscript II Preamplification System®, Invitrogen, Carlsbad, California, USA). Real time PCR was performed on a Prism 7500 sequence detection system with SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, California, USA). The 7500 sequence detector® (Applied Biosystems) was programmed with the following PCR conditions: 40 cycles of 15-second denaturation at 95° C and 1-minute amplification at 60° C.
All reactions were run in triplicate and were normalized to the housekeeping gene *HPRT* (Hypoxanthine PhosphoRibosylTransferase). The evaluation of

relative differences of real-time PCR results was calculated by using the comparative cycle threshold (*CT*) method. The primer sets of rabbit used in this study were as Table 1.

Statistical analysis

Statistical analyses were carried out using SPSS statistics 20 (IBM, NY, USA). After checking the normality, independence, and equality of variance of data, two-way ANOVA was used to compare the distance change for tooth movement and the number of osteoclasts. For evaluating the measurement error of repetitive counting of osteoclasts with a week interval, *Bland-Altman method* was used[71,72].

For the relative quantitation of mRNAs, the non-parametric Kruskal-Wallis test was used. The significance level was set at $p < 0.05$.

IV. Results

Measurement of tooth movement

Of the total 18 rabbits, one died after general anesthesia and we measured the distance of tooth movement for 17 rabbits (Table 2). When comparing changes in the distance of tooth movement, it was the greatest in Group 3 (total 5.05 mm) but there was no significant difference among the groups ($p > 0.05$) (Table 3). There was significant difference in the distance among the days of sacrifice: T1 = T2 < T3 ($p < 0.001$) (Table 3).

Semi-quantitation of osteoclasts

We counted the number of osteoclasts on the right loaded and left unloaded side for all subjects (Tables 4 and 5).

On the right side, there was no significant difference in the number of osteoclasts among groups for the incisor and first molar ($p = 0.871$, $R^2 = 0.186$). There was marginally significant difference in the number of osteoclasts among days only for the incisor: T1 > T2 = T3 ($p = 0.052$).

On the left side, there was no significant difference among groups and days for the incisor and first molar, respectively.

For evaluating the measurement error of repetitive measurement with one week interval, *Bland-Altman method* was used. Most observations were found inside the interval of limit of agreement (95% as the mean difference ± 2 standard deviation) and the repetitive measurement agreed well (Figure 5).

Relative Quantitation of mRNAs by Real Time Reverse Transcription-PCR

We quantified mRNAs of RANKL, OPG, IL-1α, IL-1β, IL-6, and TNF-α for the subjects at T2 (Tables 6 to 11). And for the subjects at T3, mRNAs of RANKL and OPG were quantified (Tables 12 and 13). Because all

reactions were repeated in triplicate, the number of subjects is three. The relative differences of real-time PCR results were evaluated by using the comparative cycle threshold (CT) method where we set the left first molar (PL) data in Group 1 as a reference value (1.0) of all other data.

For RANKL at T2, there was significant difference only in the left first molar (PL): Group 2 > 1 > 3 ($p < 0.05$) (Table 6).

For RANKL at T3, there was significant difference in the right first molar (PR): Group 3 > 1 > 2 ($p < 0.05$) (Table 12).

For OPG at T2, there was significant difference in PR and PL: PR-Group 2 > 1 > 3, PL - Group 2 > 1 > 3 ($p<0.05$) (Table 7).

For OPG at T3, sacrifice there was significant difference in PR and the left incisor (AL): PR - Group 2 > 3 > 1, AL - Group 1 > 3 > 2 ($p < 0.05$) (Table 13).

For IL-1α, IL-1β, IL-6, and TNF-α at T2, there was no significant difference in all four sites ($p > 0.05$) (Tables 8 to 11).

V. Discussion

Tooth movement and time frame

In the results the amount of total tooth movement for 14 days was the largest by a narrow margin for Group 3 (ramus osteotomy), but there was no significant difference among the three groups (Table 3). There was statistically significant difference in the amount of tooth movement among the days of sacrifice and we could observe the dramatic change from T2 to T3 in all groups (Table 3).

The time frame is important in interpreting animal study. Ren et al.[73] reported that many studies of tooth movement had been performed for various duration in a wide range of animal species such as rat (1~2 weeks), cat (4~25 weeks), rabbit (1 week), beagle dog (4~17 weeks), and monkey (8~16 weeks). In other study of rabbit, after drilling intentional socket mesial to the first molar, Yu et al.[74] reported significantly greater tooth movement at 2^{th} and 4^{th} week. Venkataramana et al.[75] found that locally administrated bisphosphonate in rabbits had shown reduced tooth movement after 3 weeks. Roberts WE suggested that rabbits' bone metabolism was 3 times faster than that of human beings[76]. Thus, in terms of the bone turnover rates in rabbits, 2 weeks would correspond to 1.5 months (6 weeks) in humans. After orthognathic surgery in humans, 6 weeks are relatively early time for orthodontic tooth movement. And as the studies above, some meaningful results came into view 2 to 4 weeks later, therefore 2 weeks can be regarded as early time in rabbit.

In our study rabbit showed dramatic response in early stage, especially from T2 to T3 regardless of groups. Although we could not make sure of the osteotomy effect on the tooth movement speed, we could have seen any difference in tooth movement among groups if we observed more than 14 days.

Osteoclast counting

There was no significant difference in the number of osteoclasts on the right loaded and the left unloaded sides for anterior and posterior teeth, however at T1 in all groups there was marginally significant difference among days only for the right incisor ($p=0.052$). : T1 > T2 = T3 (Tables 4 and 5)

Although we could not observe the effect of osteotomy on the number of alveolar osteoclasts in this early stage of tooth movement, the number of osteoclasts were visibly decreased in all groups from T1 to T3.

In cell-mediated alveolar bone resorption, the appearance of osteoclasts has been considered to be the requisite first step[42,77]. Several lines of evidence exist for the fact that the immediate precursors of osteoclasts are present in the PDL and are activated or transformed to mature osteoclasts after orthodontic mechanotherapy. Roberts and Ferguson[45] found that osteoclast numbers per unit bone surface area show a peak level about 50 hours after orthodontic force application. Additionally, new osteoclasts reach the PDL from haemopoietic organs via the blood circulation, and from alveolar bone marrow cavities, during the orthodontic treatment period, which can last 2 to 3 years[78~82]. Other study of orthodontic tooth movement in rabbits showed that significant decrease in osteoclast number at 2^{th} and 4^{th} week was followed by increased tooth movement[74].

Therefore, we suppose that as alveolar bone resorption progressed, the number of activated osteoclasts decreased gradually during early stage of 14 days in all groups, and the environment good for more tooth movement being made. And after early stage, new osteoclasts from other sources would appear and begin more alveolar bone resorption, when considering the amount of RANKL as below.

Quantification of Cytokines

There showed the greatest amount of RANKL at T3 on the mesial side of

the right first molar, which was near ramus osteotomy site (Table 12). For OPG we could not find consistent and meaningful data at T2 and T3 (Tables 7 and 13). For other four cytokines there was no significant difference among groups at T2 (Tables 8 to 11).

Bone remodeling processes, which are involved in orthodontic tooth movement, are regulated by the activation and interaction of osteoclasts and osteoblasts. Those cells are known to be controlled by various cytokines, such as IL-1, TNF-α, IL-6 and RANK/RANKL/OPG signaling axis which are the keys to the cellular response[38,42,63~67].

Especially RANKL and its receptor RANK expressed on osteoclasts and their precursor cells turned out to be the molecular determinants of osteoclast formation and function[83]. Cell to cell signaling by RANKL is essential for the induction of osteoclast differentiation. IL-1, TNF-α, and IL-6 are known to stimulate bone resorption by their ability to upregulate RANKL expression by osteoblasts/stromal cells.

Another cytokine, OPG also produced by osteoblasts/stromal cells acts as an inhibitor of osteoclast function by competing with RANKL for the membrane receptor RANK[37]. Evidence is emerging which suggests that RANKL and OPG produced by PDL fibroblasts and osteoblasts play important roles in regulating connective tissue turnover and bone resorption during orthodontic tooth movement[39,84~88].

Therefore we suppose that in early stage of tooth movement, which was 14 days in our study, RANKL played a major role in compression side, and OPG played a minor role everywhere. Although there was no significant difference for tooth movement in early stage, fracture healing in surgery site seemed to already apply positive impact on the right molar side adjacent to the site in molecular level. Therefore we might see some difference in the number of osteoclasts and tooth movement among groups if we observed to late stage more than 14 days.

How ramus osteotomy affects tooth movement

Fracture healing at the osteotomy site close to or somewhat far from the loaded tooth has much in common with orthodontic tooth movement in the way that goes through the similar cellular and molecular reactions. Various cytokines such as IL-1, TNF-α, IL-6, and RANK/RANKL/OPG are also the key to the cellular response [9,16,17,18].

When to move the tooth immediately after corticotomy and mucoperiosteal flap surgery adjacent to the tooth, the sites of response to orthodontic force and those surgery overlap each other. And it can be supposed that the RAP caused by the surgeries has a great synergy effect on tooth movement resulted from mechanical stimulus[1,2,3,7,25,26].

However how surgery distant from the loaded tooth has an effect on tooth movement in human and rats[8,69,70] cannot be logically explained at cellular and molecular level. Whether there is any communication mechanism between two different sites or how it works if any, is not identified.

As mentioned earlier in review of literature, there are a few reports of obvious interaction and association between two distant sites of bone remodeling.

Teixeira et al.[69] reported that small perforations of cortical bone 4mm distant from tooth in rats increased the rate of bone remodeling and tooth movement. They demonstrated that the increase in bone remodeling rate was not only limited to the area of the loaded tooth, but also extended to the adjacent tissues surrounding tooth, and the perforations do not need to be close to the tooth to be moved to accelerate the rate of movement.

In other study, local healing process of the bur hole defect in the rat tibia resulted in a significant increase in mineralizing surface, mineral apposition rate, and bone formation rate in both femora and the fourth lumbar vertebra[70]. It was suggested that the regional enhancement of bone formation result in a systemic impact on bone metabolism, that is a systemic acceleratory phenomenon (SAP) accompanied with the RAP.

And other clinical study in human reported that after orthognathic surgery, RAP activated in fracture healing accelerated the tooth movement[8]. This clinical study showed that the tooth movement was accelerated and the serum alkaline phosphatase and C-terminal telopeptide of type I collagen (ICTP) level, which is a bone resorption metabolite of type I collagen in bone, significantly increased in the first week to the third month postoperatively. The authors suggested that the phenomenon of postoperatively accelerated orthodontic tooth movement was because of the increase in osteoclastic activities and metabolic changes in the dentoalveolus caused by orthognathic surgery.

If we suppose that any mechanism such as SAP works in the experimental condition like our study, various hormones and humoral factors can be regarded as the vehicle[73].

Most, if not all, calciotropic hormones have been shown to upregulate messenger RNA expression of RANKL in osteoblast cell lines and primary cell cultures[89,90].

They include 1,25$(OH)_2$ vitamin D3 and parathyroid hormone(PTH)-related peptide (PTHrP)[91], which are the major calciotropic factors that are known to induce increases in bone resorption.

Humoral factors that decrease bone resorption and increase density, such as estrogens, have a converse effect on the coupling between the osteoblast and osteoclast — OPG expression is increased and/or RANKL expression is decreased, leading to decrease in RANK activation and subsequently the numbers of activated osteoclasts in the bone. Recently the cytokine thrombopoietin, which regulates platelet levels, has also been shown to induce OPG expression in animals, leading to abnormal increases in bone density[92].

Although how calciotropic hormones and humoral factors affect different site was not exactly known, the RANK signaling pathway of osteoclasts in the

alveolar bone is supposed to integrate diverse humoral signals that regulate bone resorption and calcium homeostasis.

Although the results in this study cannot be directly applied to human, we suppose to get some impression of the increase in tooth movement speed during a few months after orthognathic surgery in human. Further studies of longer period after early stage are needed to establish the possible mechanism called RAP and SAP through various methods such as blood test and genetic analysis.

VI. Conclusions

1. The amount of total tooth movement during 14 days was the largest for Group 3 (ramus osteotomy), but there was no significant difference among three groups ($p > 0.05$). In all groups, there was significant difference among days ($p < 0.05$). : T1 = T2 < T3

2. There was no significant difference in the number of osteoclasts on the right and left sides for the incisor and first molar teeth among groups. But at T1, there was a marginally significant difference among days in all groups: T1 > T2 = T3 ($p = 0.052$).

3. Ramus osteotomy group (Group 3) showed a significant difference in the amount of RANKL on the mesial side of right posterior first molar, which was adjacent to the ramus osteotomy site, at T3 ($p < 0.05$).

4. The present study suggests that the orthognathic surgical regimen (mandibular ramus osteotomy) has some influences on molecular change in alveolar bone adjacent to the osteotomy site at an early stage of tooth movement in a rabbit model.

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Figure 1. Nickel Titanium closed coil spring was connected only between the right incisor and first molar.

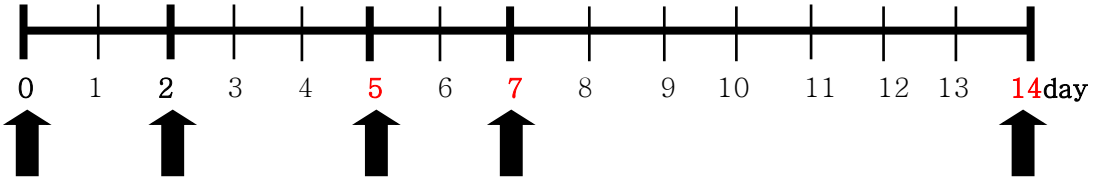

Figure 2. Measurement of tooth movement

0 day: Connection with NiTi closed coil spring in each subject

2 day: Sham operation or vertical ramus osteotomy

5 (T1), 7 (T2), and 14 (T3) days: Sacrifice

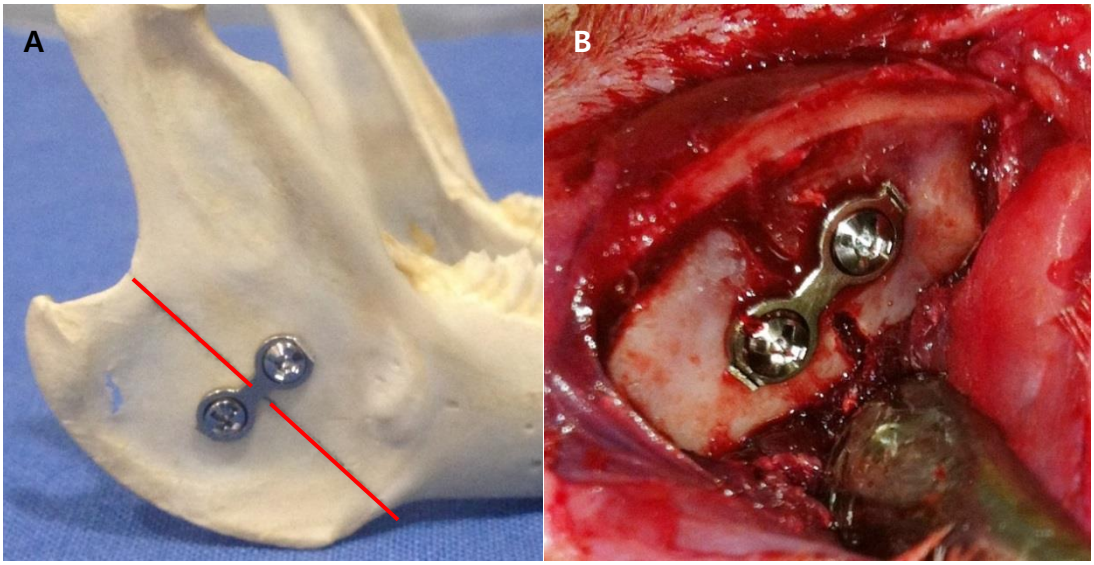

Figure 3. Mandibular ramus osteotomy: A, the osteotomy (red line) from post-condylar notch to the postero-inferior area of the mandibular body in parallel with the anterior border ; B, the separated bones fixed in the middle of the osteotomy line with 2-hole mini-plate and two mini-screws.

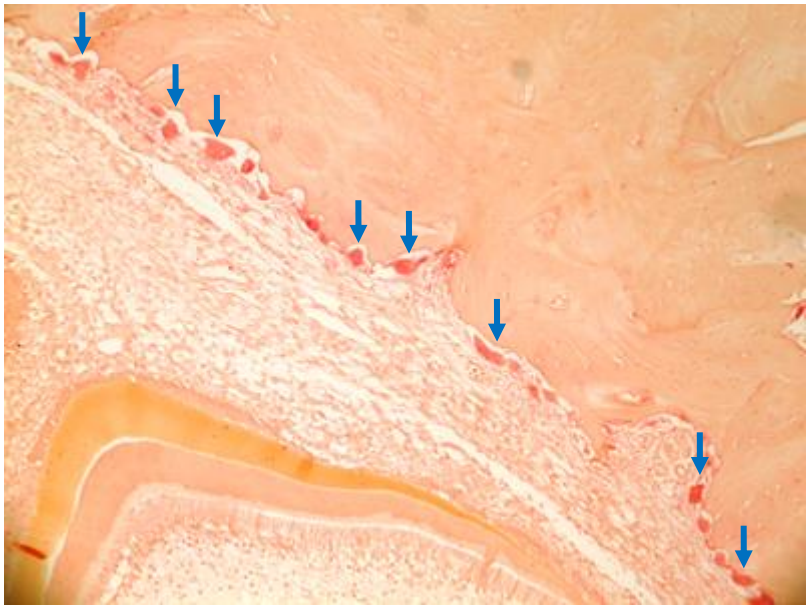

Figure 4. Semi-quantitation of osteoclasts

All the multinucleated cells (blue arrows), which were stained by acid phosphatase substrate made from incubating solution and in touch with alveolar bone, were counted selectively (×100).

Figure 5. For evaluating the measurement error of repetitive measurement with one week interval, *Bland–Altman method* was used. The most observations were found inside the interval of limit of agreement (95% as the mean difference ± 2 standard deviation(SD)) and the repetitive measurement agreed well.

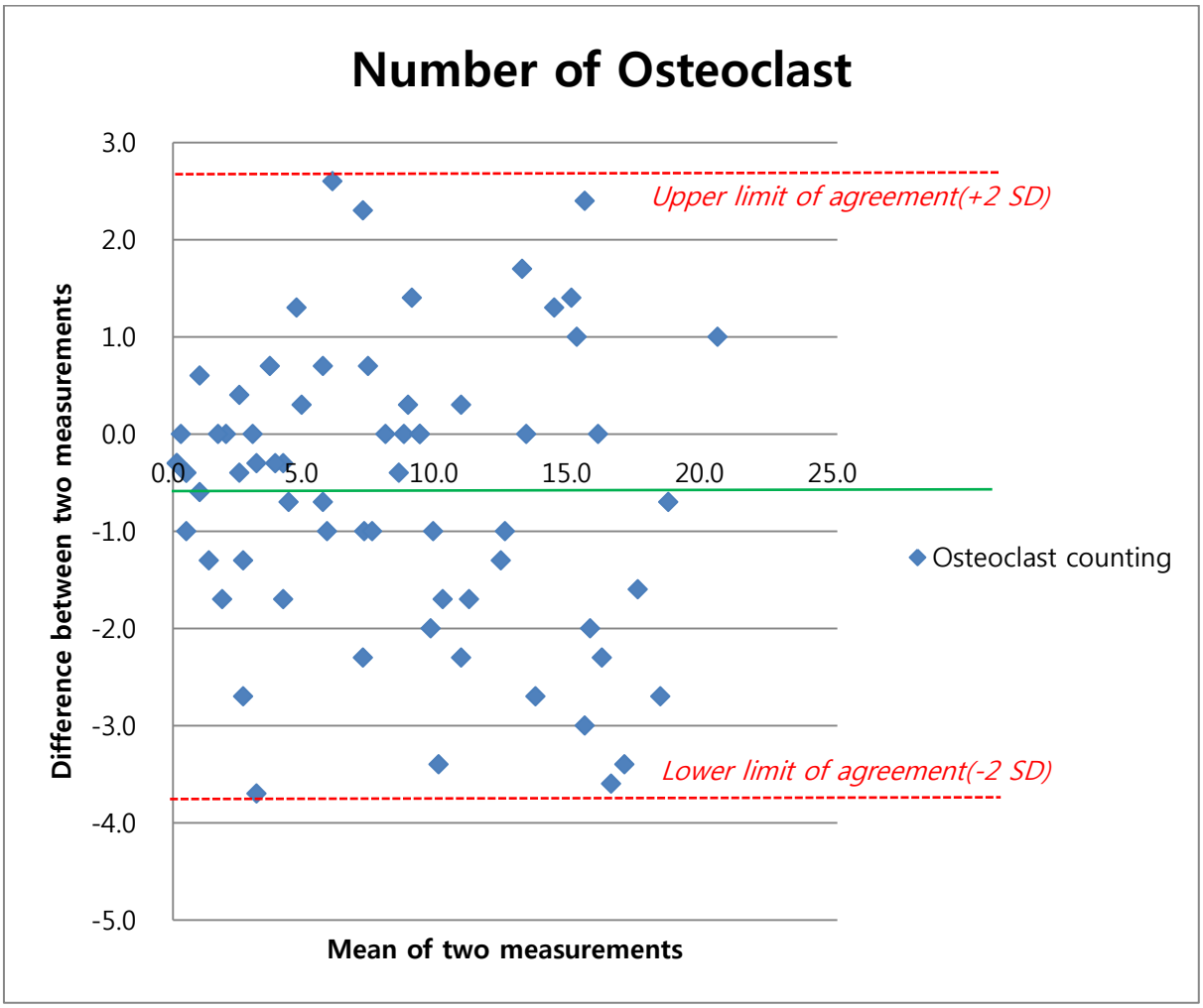

Table 1. The primer sets used in this study

Cytokines	Direction	Primer sequences
RANKL	*forward*	5'-AGCACGCGCTGCTTCTACAG-3'
	reverse	5'-GTGAGGTGAGCAAACGGCTG-3'
OPG	*forward*	5'-AAAGCCCCCTGTCGAAAACA-3'
	reverse	5'-TCCAAGGTGAGGTTTGCGTG-3'
IL-1 α	*forward*	5'-CTGAGTACCTCTGAAACCTCTG-3'
	reverse	5'-GGAACTGATCTGGGCTTGATG-3'
IL-1 β	*forward*	5'-CCAGTGAGATGATGGCTTACC-3'
	reverse	5'-GGAAGCTCTTGTTGTAGGGTTG-3'
IL-6	*forward*	5'-CAAGGAGCTGAGGAAAGAG-3'
	reverse	5'-GATACATCCGGAACTCCATC-3'
TNF α	*forward*	5'-CTCAGCCTCTTCTCTTTCCTG-3'
	reverse	5'-GATACATCCGGAACTCCATC-3'
HPRT	*forward*	5'-CCTTACCTTCAAGGACGTTC-3'
	reverse	5'-GTAATCAGGCGAGTGCTAAG-3'

Table 2. Measurement of tooth movement at each day (unit: mm)

	Initial distance	Second distance at sacrifice		
Group-subject	0^{th} day	T1	T2	T3
1-A	*29.0*	*28.0*	-	-
1-B	*31.0*	*29.0*	-	-
1-C	*29.0*	-	*27.0*	-
1-D	*29.0*	-	*28.5*	-
1-E	*29.5*	-	-	*26.5*
1-F	*28.0*	-	-	*25.5*
2-A	*29.5*	*27.5*	-	-
2-B	*31.5*	*30.5*	-	-
2-C	*27.5*	-	*26.0*	-
2-D	*27.0*	-	*26.0*	-
2-E	*29.0*	-	-	*27.0*
2-F	*28.5*	-	-	*
3-A	*28.0*	*26.5*	-	-
3-B	*28.5*	*28.0*	-	-
3-C	*29.0*	-	*28.0*	-
3-D	*26.5*	-	*26.0*	-
3-E	*32.5*	-	-	*29.0*
3-F	*30.5*	-	-	*27.5*

*: One rabbit (subject F in Group 2) died after general anesthesia and the data was lost.

Table 3. Description of tooth movement (unit : mm)

Group	n	initial distance mean(SD)	Amount of tooth movement			
			T1 mean(SD) n	T2 mean(SD) n	T3 mean(SD) n	Total change of distance
Group 1	6	29.1(1.1)	1.5(0.7)[a*] 2	0.75(0.4)[a] 2	2.75(0.4)[b] 2	5.0
Group 2	5	28.9(1.8)	1.5(0.7)[a] 2	1.25(0.4)[a] 2	2.0(−)[b] 1	4.75
Group 3	6	29.2(2.1)	1.0(0.7)[a] 2	0.75(0.4)[a] 2	3.3(0.4)[b] 2	5.05

*: Different alphabets mean significantly different at type one error rate 0.05.

There was no significant difference for groups ($p = 0.982$), but there was significant difference for days ($p < 0.001$) (T1 = T2 < T3). $R^2 = 0.732$

Table 4. Number of osteoclast at the right sides (experimental side)

	T1			T2			T3		
	n	Ant	Post	n	Ant	Post	n	Ant	Post
Group 1	2	14.7	8.4	2	13.2	16.2	2	12.5	9.5
Group 2	2	17.2	14.5	2	15.4	10.4	1	10.7	8.7
Group 3	2	17.5	11.9	2	15.5	15.3	2	9.0	12.0

Ant (anterior tooth)

There was no significant difference among groups ($p = 0.871$, $R^2 = 0.213$).

Post (posterior tooth)

There was no significant difference among groups and days ($R^2 = 0.186$).

In all groups, there was marginally significant difference among days ($p = 0.052$): T1 > T2 = T3.

Table 5. Number of osteoclasts at the left sides (control side)

	T1			T2			T3		
	n	Ant	Post	n	Ant	Post	n	Ant	Post
Group 1	2	4.5	1.2	2	6.9	1.3	2	6.7	3.2
Group 2	2	6.9	1.5	2	3.2	2.3	1	3.3	2.7
Group 3	2	4.0	2.7	2	4.5	2.5	2	4.7	4.0

Ant (anterior tooth)

There was no significant difference among groups and days, respectively.

Post (posterior tooth)

There was no significant difference among groups and days, respectively.

Table 6. Mean levels (SD) of RANKL at T2 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	0.70(0.55)	0.63(0.25)	1.84(0.45)	1.00(ref)
2	1.20(0.59)	0.87(0.50)	2.18(0.93)	1.74(0.42)
3	0.34(0.75)	0.66(0.29)	0.57(0.27)	0.33(0.07)
p-value*	0.148	0.875	0.061	0.024

*: Kruskal-Wallis test was performed.

AR : right anterior tooth

AL : left anterior tooth

PR : right posterior tooth

PL : left posterior tooth

Table 7. Mean levels (SD) of OPG at T2 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	0.67(0.14)	1.86(0.45)	1.24(0.24)	1.00(ref)
2	4.74(1.07)	2.58(1.08)	2.74(0.38)	8.83(2.42)
3	0.56(0.13)	0.59(0.34)	0.50(0.03)	0.52(0.24)
p-value*	0.051	0.051	0.027	0.027

*: Kruskal-Wallis test was performed.

AR : right anterior tooth

AL : left anterior tooth

PR : right posterior tooth

PL : left posterior tooth

Table 8. Mean levels (SD) of IL-1α at T2 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	1.53(0.36)	19.36(6.29)	9.62(3.00)	1.0(ref)
2	8.03(10.34)	0.39(0.26)	3.37(1.39)	0.55(0.17)
3	0.48(0.12)	9.51(14.69)	34.17(56.17)	0.67(0.34)
p-value*	0.061	0.061	0.288	0.240

*: Kruskal-Wallis test was performed.

AR : right anterior tooth

AL : left anterior tooth

PR : right posterior tooth

PL : left posterior tooth

Table 9. Mean levels (SD) of IL-1β at T2 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	3.33(0.80)	4.31(1.82)	2.37(0.54)	1.00(ref)
2	0.41(0.33)	0.10(0.10)	0.15(0.09)	1.11(0.57)
3	0.19(0.10)	0.05(0.05)	0.06(0.03)	0.14(0.07)
p-value*	0.051	0.061	0.051	0.055

*: Kruskal-Wallis test was performed.

AR : right anterior tooth

AL : left anterior tooth

PR : right posterior tooth

PL : left posterior tooth

Table 10. Mean levels (SD) of IL-6 at T2 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	3.59(2.66)	3.67(4.41)	5.79(7.64)	1.00(ref)
2	4.01(4.36)	1.17(1.49)	1.06(1.30)	2.90(3.40)
3	2.74(3.00)	0.65(0.78)	0.95(0.86)	0.76(0.81)
p-value*	0.733	0.202	0.252	0.479

*: Kruskal-Wallis test was performed.

AR : right anterior tooth

AL : left anterior tooth

PR : right posterior tooth

PL : left posterior tooth

Table 11. Mean levels (SD) of TNF-α at T2 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	1.60(1.62)	6.04(3.38)	37.80(64.07)	1.00(ref)
2	0.37(0.09)	0.35(0.25)	0.93(0.69)	6.19(4.70)
3	0.26(0.18)	0.24(0.23)	0.39(0.43)	0.58(0.37)
p-value*	0.061	0.051	0.193	0.055

*: Kruskal-Wallis test was performed.

AR : right anterior tooth

AL : left anterior tooth

PR : right posterior tooth

PL : left posterior tooth

Table 12. Mean levels (SD) of RANKL at T3 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	0.48(0.11)	−[@]	0.97(0.07)[a]	1.00(−)[&]
2	1.57(0.21)	0.59(0.06)[b]	0.28(0.04)[b]	0.29(0.06)
3	2.04(0.73)	1.88(0.19)[c]	6.52(1.42)[c]	1.06(0.16)
p−value[*]	0.061	−[@]	0.027	0.055

[*]: Kruskal−Wallis test was performed.

[#] Different alphabets mean significantly different at type one error rate 0.05.

[&] Reference group: Excluded from the analysis

[@] There was unknown error in group 1(HPRT data).

Table 13. Mean levels (SD) of OPG at T3 according to groups and sites (n=3)

Group＼site	AR	AL	PR	PL
1	0.09(0.01)	1.36(0.58)[a#]	0.02(0.002)[a]	1.00(−)[&]
2	0.12(0.03)	0.10(0.01)[b]	0.48(0.02)[b]	0.13(0.03)
3	0.11(0.05)	0.33(0.09)[c]	0.25(0.11)[c]	1.37(0.35)
p−value[*]	0.393	0.027	0.027	0.05

[*]: Kruskal−Wallis test was performed.

[#] Different alphabets mean significantly different at type one error rate 0.05.

[&] Reference group: excluded from the analysis

국문초록

하악지 골절단술 이후의 초기 치아이동에 관한 동물 연구

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이번 연구의 목적은 하악지 골절단술이 이 부위로부터 멀리 떨어진 부위의 초기 치아 이동에 영향이 나타나는지를 살펴보고자 하는 것이다.

뉴질랜드산 흰 토끼 수컷 18마리(몸무게 4.0 kg)를 6마리씩 세 군으로 분류하였다. 1군은 수술 없이 치아이동만 시행하고, 2군은 sham 수술로 연조직 절개만 하고 치아이동을 하였으며 3군은 하악지 골절단술과 함께 치아이동을 하였다. 니켈-티타늄 폐쇄용 코일 스프링을 사용하여 토끼의 우측 절치와 우측 제1대구치를 상호 견인하면서 거리 변화량을 계측하였다. 5 (T1), 7 (T2), 14일째 (T3)에 각 군당 2마리씩 희생하여 압박측인 우측과 교정력을 가하지 않은 좌측에서의 절치 원심과 제1대구치들 근심의 치조골 수직 절단면 조직 표본에서 치조골과 접촉해 있는 파골 세포 수를 계측하였으며, T2 시기 희생군에 대해서는 RANKL, OPG, IL-1α, IL-1β, IL-6 및 TNF-α 등과 T3 시기 희생군에 대해서는 RANKL과 OPG 를 정량분석하기 위해 좌우 네 부위의 치조골 수직 절편에 대해 실시간 역전사 중합효소연쇄반응(real time reverse transcription polymerase chain reaction) 을 시행하였다.

그 결과는 다음과 같았다.

1. 치아 이동량에서는 3군에서 근소하게 최대량을 보였으나(총 5.05mm) 통계적으로 유의하지 않았다.

2. 파골세포의 수는 우측의 절치와 제1대구치에서 군별로 유의한 차이가 없었다. 하지만 모든 군의 우측 절치와 제1대구치에서 T1에 한계적으로 유의한 증가를 보였다 ($p = 0.052$). 좌측 (대조측) 에서는 군별 및 날짜에 대해 절치와 제1대구치에서의 유의한 차이는 없었다.
3. RANKL은 3군에서 T3에는 골절단술 부위에 근접한 우측 (실험측) 제1대구치 부위에서 높은 수준으로 관찰되었다 ($3 > 1 > 2$ 군, $p < 0.05$).
4. OPG 는 모든 군에서 T2와 T3에 일관된 결과를 보이지 않았다.
5. IL-1α, IL-1β, IL-6 및 TNF-α 는 네 부위 모두에서 T2에 군별로 유의한 차이를 보이지 않았다.

이번 토끼 연구를 통해 하악지 골절단술은 치아 이동 초기 단계에서 인근의 치조골 부위에서 분자생물학적인 수준의 변화가 일어나도록 영향을 끼치는 것으로 생각할 수 있겠다.

주요어 : 하악지 골절단술, 치아이동, 파골세포

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